EFFECT OF UNILATERAL MONOCHROMATIC LIGHT AND GROUP ORIENTATION ON THE POLARITY OF GERMINATING FUCUS SPORES

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(WITH TWO FIGURES)

Introduction

The power of light stimuli to produce orientations and tropisms is a phenomenon which has been widely demonstrated in both the plant and animal kingdoms. Not only can unilateral illumination direct movements and growth, but in some species of plants, namely, *Equisetum*, *Fucus*, *Puccinia*, and related forms, natural white light has been found to establish the direction of the first cleavage plane of the germinating spore. Since in such cases the cell on the shaded side of the spore becomes the rhizoidal cell, the polarity of the plant is determined by light, irrespective of gravity. The primary purpose of the present investigation was to determine whether all wave lengths of light, the intensity factor being eliminated, are able to bring about this orientation and establish the polarity of the germinating spores of *Fucus inflatus*. Subsidiary studies which have been made in this connection with interesting results are (1) on that most interesting and little known phenomenon which I have called "group orientation," consisting in the orientation of the cleavage plane and the establishment of the apical and basal ends of the dividing spore by the direction of some other spore or group of spores in close proximity; and (2) on the phototropisms of the young rhizoids in monochromatic lights of equal intensities.

In reviewing the literature on biological experiments with monochromatic light, one is struck by the small number of quantitative records of the quality and especially of the intensity of the illuminations used. The ordinary light filters used to obtain monochromatic light transmit not only those wave lengths which predominate and give the color to the screen, but also other parts

of the spectrum, the presence of which can be detected only by a spectroscopic analysis. For example, certain results are frequently ascribed to blue light, with no record of just what range of the spectrum is used, nor what wave lengths other than the predominating ones are acting.

Another source of inaccuracy has been the neglect or oversight of the great variation in the intensity or quantity of radiant energy transmitted by the color screens. Biological experimenters for the most part have failed to take into consideration the fact that the quantity as well as the quality of the light stimulus varies with the different colors, and that the former variable must be eliminated before results can be attributed to differences in wave length alone. In some cases the importance of differences in the intensity factor has been recognized, but no method was known to the writer whereby the different colored lights could be compared as to their amounts of radiant energy (10).

There have been, of course, several methods devised by means of which the relative intensity of monochromatic lights can be measured. The first investigation in which the attempt was made to get monochromatic light of known wave length and equal energy was that of BLAAUW (1). He used glass color screens, and states that the lights transmitted were equal in intensity when measured with a Weber photometer. The accuracy of this method can be seriously questioned.

The next exact work of this nature was done by KNIEP and MINDER (11). They used a blue and a red color screen, and a green solution, with sunlight as the source of light. The wave lengths to which each was transparent were known; and the energy behind each was determined by means of a thermopile and d'Arsonval galvanometer. The interference of the long heat rays was prevented by inserting a water layer in a parallel-sided container between the thermopile and the source of light.

DAY (5) obtained light of known wave length by means of a spectrum from Nernst glowers, formed by a carbon bisulphide prism and cut down by a diaphragm with narrow vertical slits which could be adjusted so as to allow any desired region of the spectrum to be transmitted. In this adjustment a spectroscope

was used to determine the exact range of wave lengths passing through the slit in each of the four illuminations used (red, yellow, green, and blue). DAY measured the intensity of each with a Boys radiomicrometer, and balanced them by varying the number of glowers employed in the lamps. Thus there was one glower for the red light, two for the yellow, and three for the green and blue.

LAURENS (12), in an investigation of the reactions of amphibians, employed these same methods and the same apparatus for the quantitative analysis of the monochromatic light he used, and balanced them similarly with respect to their relative intensity. GROSS (9) also used the same methods in determining the reactions of arthropods to monochromatic light. MAST (14) measured the different effects of monochromatic light by the orientation of organisms in a field in which two differently colored beams crossed at right angles. He used spectral regions of known energy and wave length. The method of obtaining the distribution of energy is not described. PARR (15) did quantitative work on the response of *Pilobolus* to different wave lengths, using apparatus of the type employed by DAY and LAURENS.

An instrument has been devised by MACDOUGAL and SPOEHR (13) which measures the total radiant energy of any light in terms of its dissociation effect on a photosensitive substance. This is measured by a galvanometer. The advantages in the use of this "photoelectric cell" are said to be its extreme sensitiveness to the wave lengths of the blue end of the spectrum, and the fact that its action in light is "more nearly that of the organism than that of any other light measuring instrument available."

There have been, therefore, three exact methods worked out for biological experiments to obtain a quantitative analysis of light stimuli, namely, those of KNIEP and MINDER, DAY, and MACDOUGAL and SPOEHR. The interesting apparatus devised by PATTEN (16), whereby a quantitative measurement of the reactions of organisms subjected to two beams of light of different intensity is obtained, might be mentioned here. The measurement is in terms of the angular deflections from an initial path of locomotion. The same methods might be applied to work with colored lights.

A quantitative measurement of the greater effectiveness of one spectral region over another of equal intensity might be measured by the angular deviation of the path of a motile organism from a line perpendicular to a line connecting the two sources.

All these methods, with the exception of those of KNIEP and MINDER, involve special apparatus often not easily available. For many problems simpler methods will accomplish the same ends. For the present investigation a method has been devised whereby color screens of known wave length transmission are used, and their relative intensities measured by means of a thermopile and galvanometer, and made equal by adjusting the distances from the light source.

Apparatus and methods

As biological science becomes more exact, with the tendency to reduce the expression of natural phenomena to mathematical formulae, it is obviously essential to define stimuli of all sorts quantitatively. Indefinite or incomplete records of light stimuli can no longer be attributed to the lack of means of measuring them, because access to a spectroscope and thermopile makes it possible to analyze any light qualitatively and quantitatively.

There are two methods of obtaining monochromatic light for biological experiments, namely, the projection of a spectrum upon the organisms, or the use of filtered light passed through a color screen. The former is theoretically the better for exact work, but technical difficulties, such as limited dispersion and low intensity, make it impractical for many investigations. Light filters of glass are the most convenient means of securing approximately monochromatic light when unilateral illumination is desired. Ordinary color screens transmit too wide a range of wave lengths for exact work, and at present there are very few whose light is of sufficient homogeneity. The best is the Wratten filter screen, which consists of a dyed gelatine film between two glass plates. MACDOUGAL and SPOEHR have described some colored glass screens designed by them for biological work, but the range of wave lengths to which each of them is transparent is considerably greater than for some of the Wratten filters.

In the experiments to be described, Wratten light filters were used, each of which was fitted as a window in the end of a dark box. Each transmitted only a narrow range of wave lengths, but together they embraced the whole of the visible spectrum. The wave lengths to which each screen was transparent were determined by testing the light transmitted by each with a direct vision spectroscope with a wave-length scale attached. Thus the quality of the light stimulus acting in each box is accurately known. The dark boxes were 10×13 cm. and 8 cm. high, in one end of each of which a hole was cut so that one of the light filters, 5×5 cm., might be fitted into it. The boxes were made light-tight with tightly fitting covers, and were painted black inside to guard against reflections within the box. The dishes used for the cultures were either the ordinary Petri dishes or special dishes made of microscope slides cemented together with zinc cement so as to make shallow oblong dishes 7.5×2.5 cm. and 1 cm. deep. It was at first deemed necessary to use such flat-sided dishes in order to prevent possible complications from reflected and refracted light in the curving sides of round dishes, but later it was found that the same results were secured in the Petri dishes. In order to expose more than one dish behind each screen so that none would be shaded by another, a rack was made to fit inside the box with cleats projecting inward from the ends so that three dishes could be slipped into it, one above the other. The light, entering through the screen at the end of the box, fell equally on the one exposed side of each of the three dishes. The rack containing the dishes could easily be lifted out and carried to the microscope for examination without disturbing the material under investigation.

The source of light first used with the filters was the electric arc. The advantage of this light over any other artificial light is that it gives all the wave lengths of the visible spectrum, so that all the filters could be used in each exposure, insuring identical conditions of temperature, constancy of illumination, etc. The disadvantages are several. In the first place, the intensity is constantly changing as the carbons burn and the arcs get longer, and the lessening of the intensity may not be the same for all the wave lengths. In the second place, fluctuations in the current

cause large variations in the intensity. In the third place, unless an automatically adjusted arc is available, it is necessary to adjust the carbons by hand every 5 to 15 minutes, and, when an 8 hour illumination is desired, this entails considerable inconvenience. The experiments were begun with this light, however, and the results on rhizoidal phototropisms in monochromatic lights made equal in intensity were obtained with it.

The spectroscopic analysis of the light passing the screens determines definitely the quality of light entering each box. It is at once evident that the quantity or intensity of light behind filters placed at equal distances from the source varies, both because the intensity of light transmitted by the different screens is different, and because the different colors are not radiated by the arc with equal intensity. This being the case, differences in results obtained behind the screens could not be attributed to differences in the quality of the light stimulus alone.

One of the simplest means of comparing the radiant energy of colored lights is by the use of the thermopile and sensitive galvanometer. The thermopile is very sensitive to the energy of any ether vibrations, whether they be the longer infra-red or so-called heat rays, or the shorter actinic rays of the spectrum. The method developed for eliminating the intensity variable in the use of the filters employed for these investigations consisted in finding the distances from the arc at which each box with its colored window should be placed in order that the intensity might be equal in each case. These distances were those at which the deflections of the galvanometer were equal when the thermopile was exposed to the arc screened by each filter in turn.

It seems necessary, on account of the questions which have been raised during the course of this work, to state that the thermopile is equally sensitive to the energy of the red and of the violet ends of the spectrum, and is, therefore, an accurate measure of the total amount of light acting behind each color screen. The difference between heat and light is only a matter of wave length. The thermopile measures light in terms of the electric current produced by the difference in temperature of the exposed and unexposed junctions; but it does so by virtue of the fact that the energy

of whatever vibrations fall upon it, be they long and therefore heating in their physiological effect, or short and therefore perceived as light, is converted into heat energy upon being absorbed by the exposed junction of the thermopile. In other words, the light of the blue end of the spectrum produces an electromotive force much less than that of the infra-red, but no less measurable.

The instruments used in the energy calibration of these screens were a Hilger thermopile with junctions of bismuth and silver, and a moderately sensitive galvanometer (d'Arsonval). An electric arc similar to the one later used in the experiments themselves was the source of light. The thermopile with the open end screened by the red filter was exposed to the light until the galvanometer indicator reached a maximum deflection, which ordinarily took about 30 seconds. The number of divisions through which the spot of light reflected from the galvanometer mirror was displaced on the scale was noted. This was repeated six times, and the average deflection recorded. The other filters were then used in turn to screen the thermopile, and thermopile and screen moved to such a distance from the arc that the displacement of the galvanometer indicator was approximately equal in each case to that produced by the red filter. This distance was also found for the thermopile when screened by a piece of clear glass, which represented the distance of the control from the source. For the experiments the quantity of light used could be varied for the whole set of screens by multiplying or dividing these distances by the same number, and the intensity in all the boxes would remain equal. The actual amount of light in meter-candles can be measured by means of a photometer. Then from the law of inverse squares, namely, that the intensity of light per unit surface varies inversely as the square of the distance from the source, the intensity at any distance from the arc can be computed.

The calibration of the set of screens was repeated seven times, or until satisfactory checks of the distances were obtained. With some thermopiles of less rapid action than the one used here, it is impossible to get results by waiting for the galvanometer indicator to come to a steady state. In such a case the deflections produced by exposure to the light for equal intervals of time can be compared.

A series of measurements for five second exposures agreed very well with those obtained by the other method.

The absolute intensities of the light behind the colored screens were measured with a Sharp-Millar photometer.[1] Since at the prescribed distances all are equal to the white light control, there remained only to measure the intensity of the arc at the distance of the control. For the experiments the distances obtained in the calibration were divided by four, so that the intensity of the arc with the photometer 85 cm. away was measured. It was 2050 meter-candles. This must be corrected for the absorption of the light by the glass of the filters. To get this "absorption coefficient," the intensity of a light was measured both with and without a screen of clear glass equal in thickness to that of the filters. A Lummer-Brodhun photometer was used for this determination. It was found that glass 1.5 mm. thick absorbed 12 per cent of the light falling upon it. To obtain the intensity of the lights as actually transmitted by the light filters, it was necessary therefore to take 88 per cent of the reading of the photometer (2050), which was 1804 meter-candles. Of course the light stimuli acting on organisms in water in the culture dishes were still less, owing to absorption by the glass of the dish and of the water.

TABLE I

DISTANCES AT WHICH INTENSITIES OF LIGHT FROM AN ELECTRIC ARC TRANSMITTED BY WRATTEN LIGHT FILTERS ARE EQUAL

Filter no.	Wave lengths in Ångstrom units*	Color	Distance from light in cm.	Intensity in meter-candles
70.....	6600–7000	Red	320÷4=80.0	1804
71.....	6200–6800	Red	275÷4=68.7	1804
72.....	5900–6200	Orange	230÷4=57.5	1804
73.....	5600–5900	Yellow	250÷4=62.5	1804
74.....	5200–5600	Green	280÷4=70.0	1804
75.....	4700–5200	Blue	250÷4=62.5	1804
76.....	4000–4700	Violet	250÷4=62.5	1804
Control.....	4000–7000+	White	340÷4=85.0	1804

* Professor E. P. LEWIS of the Physics Department of the University of California very kindly made these wave-length determinations.

The lack of agreement between these values and the energy curve of the spectrum is probably due mainly to the peculiar

[1] I am indebted to Mr. W. C. POMEROY of the Physics Department of the University of California for these determinations.

absorption of the filters, but also partly to the fact that they do not all transmit the same number of wave lengths.

Polarity

The power of external factors to determine the polarity of a germinating spore is, without doubt, the power to orient the spindle of the first dividing nucleus, if, as in the case of *Fucus*, that polarity is established by the direction of the first cleavage plane. The work on such orientations is very limited, and has often yielded negative results. DRIETSCH established the polarity of sea-urchin eggs by subjecting them to pressure, the spindle forming parallel to the flattened sides of the egg. A number of investigators have found that unilateral white light will establish the polarity of the spores of some of the lower plants by causing the first cleavage plane to be formed perpendicular to the direction of the incident light. Without exception the cell on the darker side of the spore becomes the rhizoidal cell, the other being apical. Equal illumination on all sides retards or prevents germination. This has been demonstrated in *Equisetum*, *Fucus*, *Ascophyllum*, *Pelvetia*, *Dictyota*, *Laurencia*, *Cystoseira*, *Anthoceros*, *Fimbriaria*, *Gymnogramme*, and *Puccinia*. It has been proved that gravity and contact cannot establish the polarity of these spores.

The first report of this phenomenon of polarity established by light is that of STAHL (**20**), who worked on *Equisetum*. He found that the first wall is formed perpendicular to light rays striking the spores on one side only, and that if all sides are illuminated by rotating the spores on a clinostat, the formation of the wall is retarded or prevented. The cell on the shaded side of the spore becomes the rhizoidal cell. In darkness the formation of the first wall follows no rule, and the rhizoids extend in every direction. STAHL refers to earlier work on *Marsilia* and *Chara* which indicates that gravity is a controlling factor in the orientation of the first division plane.

ROSENVINGE (**19**) showed that in *Fucus spiralis* there is no relation between gravity and the first division plane, nor does contact with a solid body have any effect. He got the same orientation to light in *Ascophyllum* and *Fucus* that STAHL did

with *Equisetum*, but with puzzling exceptions. Where the spores were in groups, the cell toward the interior of the group became the rhizoidal cell; and in the lower part of hanging drops the rhizoids appeared on the upper side of the spore regardless of the light direction. He concluded, therefore, that not only light but a difference in the concentration of oxygen on the two sides of the spores could determine their polarity. He says that as a result of their respiration the water in the center of the groups of spores is less rich in oxygen, with the result that the rhizoids are formed on that side. In support of this theory is the fact that although light can determine the polarity of all the species studied except *Fucus serratus*, namely, *Ascophyllum nodosum*, *Fucus vesciculosus*, *F. spiralis*, and *Pelvetia canaliculata*, their sensitivity to light differs, and the oxygen factor or internal causes produce frequent exceptions in all but *Pelvetia*. The rhizoids of the latter species are always formed on the darker side of the spore, and this is the one species in which the egg is surrounded by an oogonial wall which might prevent any of the effects of varying oxygen concentration that can act more potently than light on the spores of the other species. ROSENVINGE quotes KNY as finding that neither light, gravity, nor contact can influence the point of origin of the pollen tube from pollen grains, but that in the neighborhood of other grains the tube will be sent out from the side away from them, on which side the supply of oxygen or nutritive elements would be greater.

FARMER and WILLIAMS (6) state that if *Fucus* spores are illuminated on all sides they tend to remain spherical instead of producing a rhizoid by the elongation of one of the two cells. Again (7) they experimented with one-sided illumination, with the usual result that most of the rhizoids originated on the shaded side of the spore and the others were turned that way. The fact that some grew out at an angle to the incident light was attributed to "the character of the egg itself."

WINKLER (21) found the same orienting effect of light on the spores of *Cystoseira barbata*, but failed to find any effect of a difference in the oxygen content of the water. He also said that gravity and contact are not factors in the establishment of the polarity of the sporelings. He found that it is determined during

the first four hours of illumination and cannot be changed afterward by any change in the direction of the incident light. He concludes, therefore, that light can orient the spore only during fertilization.

PEIRCE and RANDOLPH (18) performed one-sided illumination experiments with *Dictyota*, *Dictyopteris*, *Laurencia*, and *Cystoseira*, and pointed out the certainty of the action of other factors besides light, because rhizoids are formed in the dark and in all-sided illumination. They said that although WINKLER (21) suggested the possibility of stopping germination by changing the direction of light every three hours, it could not be done with *Dictyopteris*. They emphasized the possibility of influences preceding the illumination affecting the polarity. Later, PEIRCE (17) demonstrated this same phenomenon, that is, the orientation of the first cleavage plane and the establishment of the apical and basal cells by light, with spores of *Anthoceros*, *Fimbriaria*, and *Gymnogramme*.

The work of FROMME (8) on the urediniospores of *Puccinia rhamni* is interesting because it refutes the idea that the orientation of the sporelings by light is due to the power of one-sided illumination to cause an aggregation of chloroplasts. He said that in darkness the germ tube grew from any side of the spore, but that in unilateral light the tubes almost always issued from the darker side of the spore.

In order to obtain an abundance of spores for experimental work, the following procedure suggested by Dr. N. L. GARDNER was followed. The fruiting plants of *Fucus inflatus*[2] were collected at Sausilito at low tide one day and kept overnight in damp newspapers. The next morning they were dried slightly by exposing them to the air for from 15 to 30 minutes. The fruiting tips were then cut off and submerged in sea water in the culture dishes. After about 15 minutes many eggs and sperms settled to the bottom of the dish, or could be scraped off into the water, and the piece of plant was then removed. The fact that *Fucus inflatus* is a monoecious species makes it impossible to tell the exact time of fertilization, but it occurs soon after the eggs escape from the oogonial sac into the water. The sperms at this time can be seen

[2] Although the identification of this species is not certain, it is thought by Dr. N. L. GARDNER to be most probably *Fucus inflatus*.

escaping from the antheridia and swimming rapidly around the eggs, then scattering as, presumably, one of them succeeds in entering. The first cross-wall can be seen very plainly 24 hours after the cultures are started. The mucilage accompanying the eggs causes them to adhere so firmly to the bottom of the dish that it is not necessary to use solid media to keep the sporelings from being displaced when the cultures are moved to the microscope stage for examination.

The original plan for determining which wave lengths are the effective ones in the orienting action of white light upon the germinating spores was to use the Wratten filter screens with the electric arc. With this purpose in view, the set of seven screens borrowed from the physics department were analyzed as to wave-length transmission, and the distances from the arc were found at which the dark boxes with these screens as windows should be placed to make the light intensity equal in all. After repeated failures to get the spores to germinate on account of the high temperatures produced by the naked arc at the distances at which it was necessary to place the cultures, this source of light was abandoned, as was also a 1000-watt Tungsten globe for the same reason. Neither would the spores germinate when the boxes were placed in direct sunlight, as the heating effect was too strong, especially behind the red filters. The first positive results were obtained with a mercury vapor lamp behind the blue, violet, and ultra violet screens, the violet transmitting waves of 4000–4700 Ångstrom units, the blue 4700–5200. The same effect was produced in these lights as is produced by white light, namely, the first cleavage planes formed perpendicular to the direction of the incident light, and the cell on the darker side of the spore became the rhizoidal cell. This effect was not produced behind the green, yellow, and red screens, behind which cultures were exposed at the same time. This experiment was unsatisfactory, inasmuch as it offered no way of proving that the other wave lengths were less capable of producing the phenomenon than were those of the blue end of the spectrum, because the blue light is so much more intense in this lamp than are the red, yellow, and green. The problem was then dropped for several months, during which time no

way was found of obtaining red light sufficiently intense which did not kill the spores by its heating effect.

Finally a set of three Wratten screens was obtained consisting of a red, a green, and a blue filter, which seemed less dense than those used before. The wave lengths to which each screen was permeable were determined by means of a direct-vision spectroscope. The experiment was then repeated, using the light from large north windows facing an open field. Thus on the bright summer days used for the experiments there was a maximum intensity of indirect light available which had no disastrous heating effect. Results were immediate and decisive. The light orientation of the axes of the young sporelings was as striking in the green and blue lights as in the control in white light (fig. 1), but entirely lacking behind the red screen where the germination and development proceeded just as they did in the control in darkness. Table II

TABLE II

WAVE LENGTHS OF LIGHT WHICH CAN ESTABLISH THE POLARITY OF GERMINATING *Fucus* SPORES IN UNILATERAL ILLUMINATION

Color of light	Wave lengths transmitted	Appearance of polarity in sporelings
Blue	4000–5000 6100–6300	+
Green	4800–6000	+
Red	5800–7000	−
White (control)	4000–7000	+

indicates that from the limits of the visible blue to somewhere in the green of the spectrum wave lengths of light can establish the polarity of *Fucus* plants, while from this point in the green to the boundary of the red they cannot (figs. 1, 2). Just where, in the green, light ceases to be effective could not be determined with these screens.

The intensities of these colored lights were not equal, but the red, as in any daylight source, was many times stronger than that of the green or blue, and therefore it is all the more significant that with so much greater energy the long red rays cannot produce the reactions of the shorter blue ones. We can say definitely that

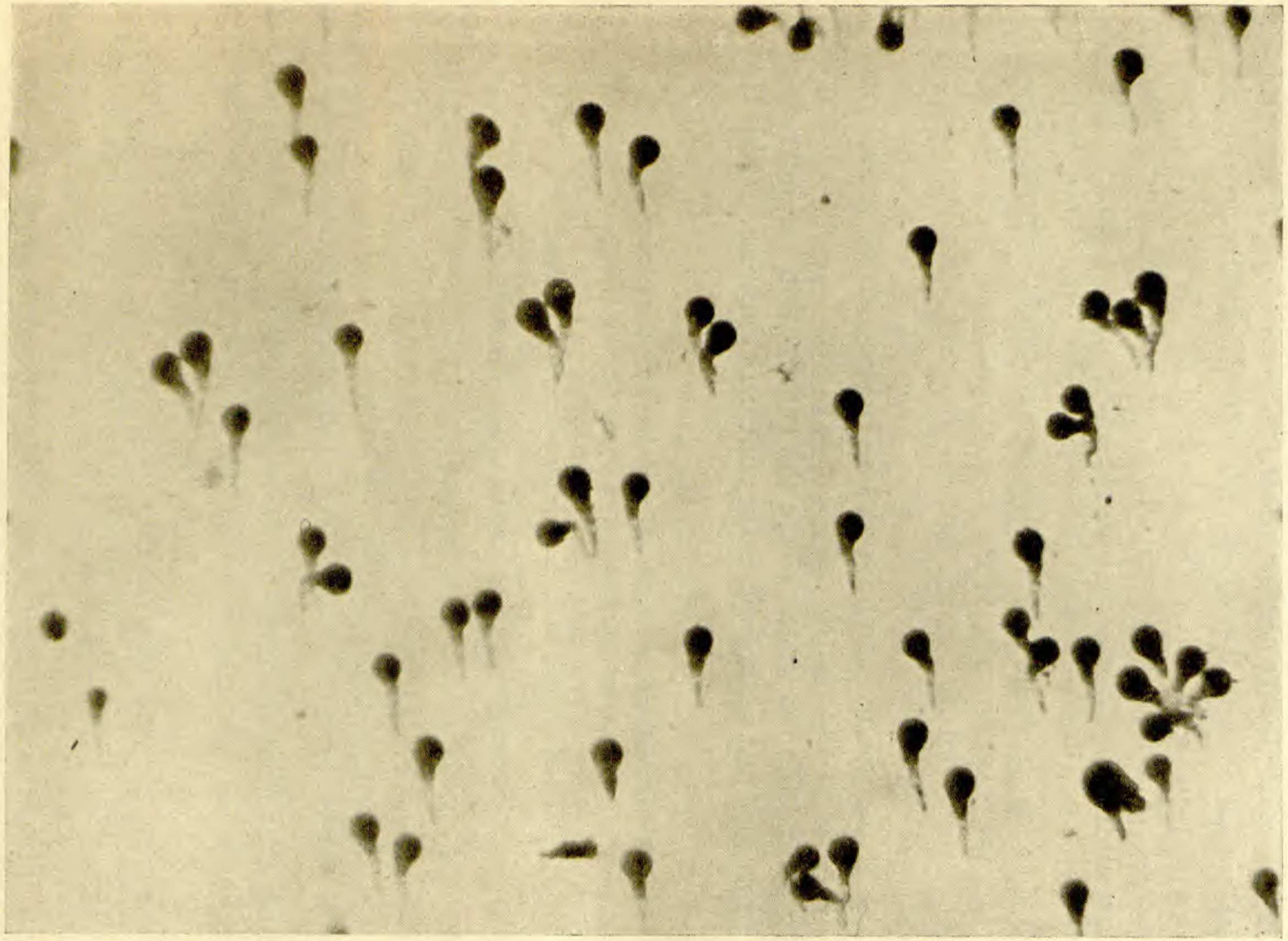

FIG. 1.—Effect of unilateral illumination with white light or its effective components on germinating *Fucus inflatus* spores, rhizoids growing from darker side of spore and extending parallel to direction of incident light; note groups of 2, 3, and 5 spores for which some chemical stimulus originating in activities of adjacent spores proved stronger than light stimulus, producing phenomenon of group orientation; ×50.

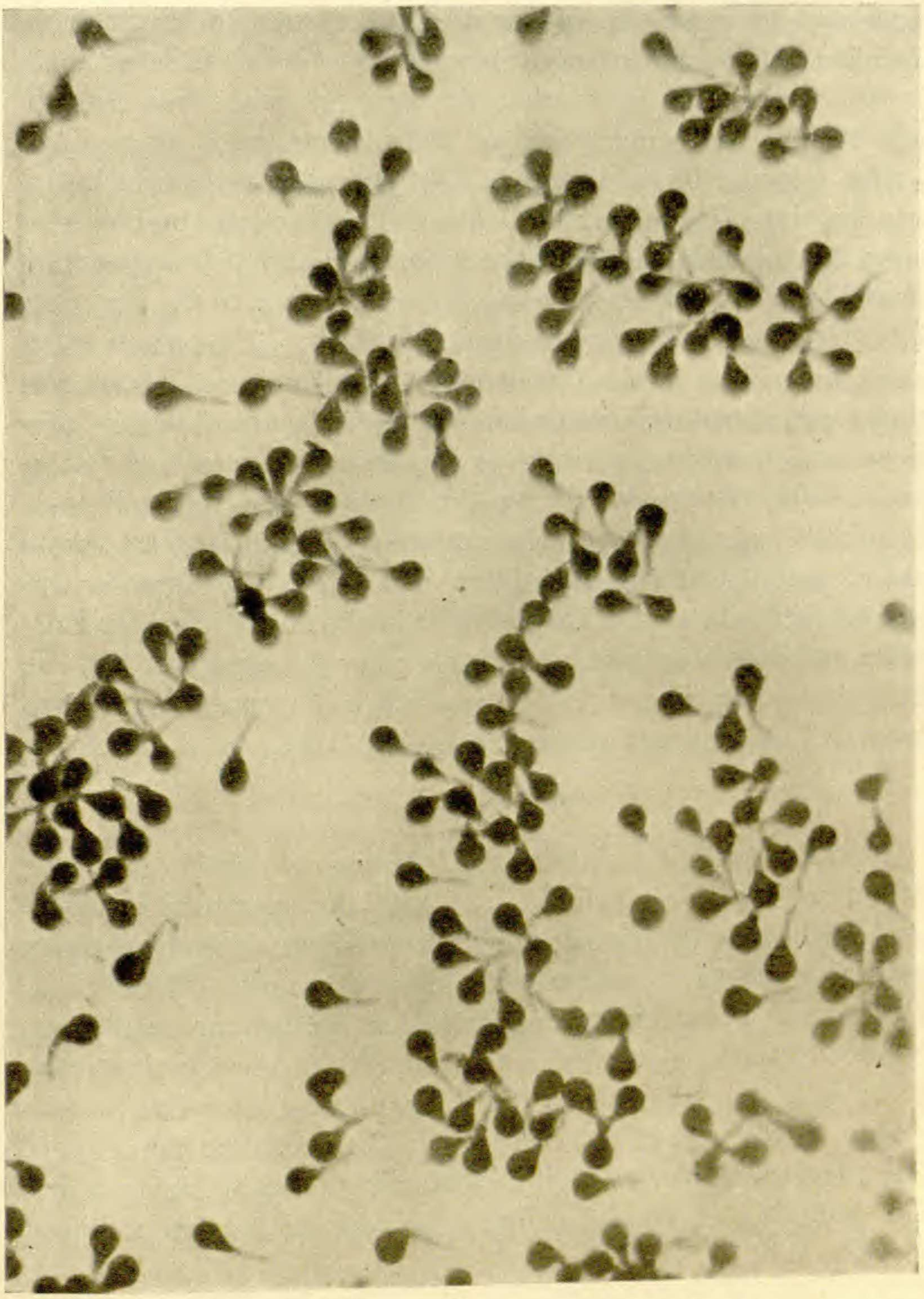

FIG. 2.—Group orientation of *Fucus inflatus* spores germinated in darkness; ×50

the power of light to orient the first cleavage planes of germinating spores and to cause the cell on the darker side to become the rhizoidal cell is dependent on the reactions within the spore initiated by the short actinic rays; that the long red rays, even though their intensity be so much greater, are ineffectual.

This response of the spores to light stimulation requires but a very low intensity of white light or its effective components. Spores left in open dishes in the laboratory rarely fail to orient themselves in a most conspicuous way with respect to the direction of the windows, nor do they require bright light. The phenomenon is just as evident in the more dimly lighted cultures left on the tables farthest removed from windows. Every culture showing this orientation, however, has more or less frequent exceptions to the general rule. Every worker on this problem has reported such exceptions, and they have been explained by assuming the existence of an inherent polarity, which as a rule is overcome by the stronger light stimulus. The fact that in absolute darkness germination and normal growth are as rapid as or more so than in light also points to an inherent polarity which is evidenced only in the absence of the stronger orienting agents.

Phototropism

WINKLER (21) first showed that the young rhizoids of *Fucus inflatus* are negatively heliotropic. With the apparatus designed for the light polarity experiments just described, several questions concerning this phenomenon were easily answered. These were: (1) what wave lengths are responsible for the turning away from a source of white light? (2) is the intensity of the illumination a factor, or is the phenomenon controlled only by the wave lengths acting; in other words, what is the rôle of the quality factor apart from the quantity of the light stimulus? (3) do all lights which have any effect at all produce the same negative tropism produced by white light?

Although the light emitted by the electric arc was too low in intensity after passing through the dense filters to establish the polarity of the young *Fucus* plants, it was noticed very early in the investigation that behind the blue and violet filters this light

was strong enough to cause all the rhizoids to turn sharply away from it. Experiments were started, therefore, to determine whether those behind the filters transmitting the longer wave lengths would not also show this effect, the intensity of all the lights being kept equal. The spores were germinated in small dishes in darkness, and allowed to grow until the rhizoidal cell had divided at least once. Cultures were then placed in the seven dark boxes behind the original set of seven Wratten filters, and these boxes placed at such distances from the naked arc that the intensity of light behind each was 1804 meter-candles (table I). The illumination was continued 6–7 hours. The next day the cultures were examined to see which ones showed the characteristic negative phototropism. It was found in every case that only those illuminated by the blue and violet light had been so affected, those behind the other filters having their rhizoids unbent, continuing in the direction in which they had started, just as did the control in darkness. With all the intensities the same (1804 meter-candles), therefore, wave lengths capable of producing the negative phototropism so commonly seen after white light illumination are those of 4000–5200 Ångstrom units, all the others having no effect.

TABLE III

WAVE LENGTHS WHICH WITH AN INTENSITY OF 1804 METER-CANDLES PRODUCE A NEGATIVE PHOTOTROPISM IN *Fucus* RHIZOIDS

Filter no.	Color	Wave lengths in Ångstrom units	Distance from arc in cm. at which intensities are equal	Appearance of negative phototropism
70.....	Red	6600–7000	80	−
71.....	Red	6200–6800	68	−
72.....	Orange	5900–6200	57	−
73.....	Yellow	5600–5900	62	−
74.....	Green	5200–5600	70	−
75.....	Blue	4700–5200	62	+
76.....	Violet	4000–4700	63	+
Control.....	White	4000–7000	85	+

The same experiment was tried with sunlight as the source of illumination. The young plants were exposed behind the filters all day in a south window. The same results were obtained as

when the arc was used. Then the experiment was repeated with diffused light. The boxes were placed in an east window for 8 hours. Again the rhizoids in the blue and violet light showed the response, but in addition a considerable but much smaller number were affected in the same way behind the green filter. Later the experiment was repeated with the second set of three filters with which results were obtained in the experiments on light polarity. These, as explained before, were less dense than the first set, and were used with the light from a north window. Here the negative phototropism appeared behind the green and blue filters, but not behind the red. Thus the same filters whose light was found to have the power of establishing the polarity of the germinating spores were also the ones which produced the rhizoidal phototropism (table II). As explained previously with reference to these filters, the intensity of the ineffective red was many times greater than the shorter but more powerful wave lengths. Just where between 4800 and 6000 Ångstrom units the rays cease to be effective it is impossible to tell with these filters, but with the first set it was found that the phototropism occasionally occurred behind the green filter with a range of 5200–5600 Å.U. It is probable, therefore, in view of the fact that in these latter experiments the blue and violet rays of 4000 to 5200 Ångstrom units never failed to produce the phenomenon at equal or less intensities, that the tropic power of light gradually decreases from the violet and blue toward the red end of the spectrum, losing its power at ordinary intensities somewhere around 5600 Å.U. It remains to be seen whether the still longer rays can be made to produce the same effects by increasing their intensity.

Only the growing tips of the rhizoids are sensitive to light. This usually results in a sharp angular turn if the direction of illumination is changed through 90° or 180°. As pointed out by LOEB and others, such tropisms are probably due to the difference in the speed of the chemical reactions going on in the two sides of the growing tip. The first protuberance of the germinating spore is not affected by light striking it from the side; and if it is so illuminated during the early stage of elongation of this cell, the first bend occurs at the cross-wall separating it from the next

rhizoidal cell. In other words, the direction of the first protuberance is at right angles to the first cleavage plane, whatever the direction from which it is subsequently illuminated. After the first cross-wall has formed in the rhizoid, however, a change in the direction of illumination results in an angular bend at the tip.

Group orientation

The preceding experiments have demonstrated the power of light to establish the polarity of germinating spores of *Fucus inflatus*. Yet another factor was found to exert an orienting influence on the spindle no less potent than that of light, that is, the proximity and direction of other germinating spores. ROSENVINGE (**19**) described this most striking and interesting phenomenon in other species of *Fucus* and in *Ascophyllum*. The first cross-wall forms perpendicular to the direction of the adjacent spore or group of spores, or, if the spore be one of a group, perpendicular to the direction of the center. The cell toward the interior of the group, or toward the source of the stimulus in the case of more isolated cells, becomes the rhizoidal cell (fig. 2). For want of a better term I have called this phenomenon *group orientation*. It is best studied in cultures germinated in darkness, and hence free from orienting effects of light.

This phenomenon is as conspicuous in groups of 2, 3, or 4 eggs as in masses of 50 or 100, so long as they are within the distance of each other through which the stimulus is effective. This distance is usually 0.2–0.3 mm., but occasionally spores as much as 0.5 mm. apart have shown the mutual orienting influence. If there are only two spores concerned, the first cleavage planes are often parallel, and the rhizoids, growing from the inner cells, meet tip to tip. In the small groups of five or six the rhizoids, all growing toward the interior, make rather symmetrical starlike designs. In the larger groups or masses of spores the phenomenon is made evident by the fact that no rhizoid is ever found taking a direction away from the group. Although the finding of many groups of eight lying together just as they escaped from the oogonial sac, and beautifully oriented with respect to each other, suggests an inherent polarity established by the relative positions of the eggs in the

oogonium, the phenomenon appears just the same if these groups are stirred up with the point of a needle before being germinated, so that the original positions are entirely changed.

The direct cause of such orientations is probably the same as that responsible for those produced by light. At least the results of the stimulations are identical, and it seems probable that the ultimate factor is the relative rate of the oxidations proceeding along an axis of the spore. It follows that the energy of light might determine this oxidation gradient in unilaterally illuminated cultures, but in those germinated in darkness the oxygen content of the water on the different sides of the spore, being more exhausted on the side next to other growing spores, might disturb the equilibrium and produce the same sort of a gradient. ROSENVINGE advanced the theory that the phenomenon was produced by a difference in the concentration of oxygen or of nutritive substances on the two sides of the spore. He thought the rhizoid forms on the side toward the center of a group or toward another egg, because as a result of the latter's metabolism the water on that side is less rich in the active substance than on the outer side of the spores. WINKLER, however, working with *Cystoseira barbata*, found that a difference in oxygen concentration which he produced artificially had no such effect on the spores.

This phenomenon, group orientation, is found in cultures germinated in light as well as those in darkness, although not so conspicuous in the former, because the light may be the stronger stimulus for many spores which in its absence would be affected by the orienting stimulus from adjacent spores. Yet the fact that it is always found in cultures germinated in unilateral light, although limited to those spores and groups of spores within a very short distance of each other, shows that within this distance the influence of neighboring spores is stronger than that of light, at least of lights with the intensities of those used in these experiments. In other words, no light was powerful enough to overcome for the more closely grouped spores the chemical stimuli originating in themselves. The relative number of spores oriented by light depends therefore on the intensity and wave-length composition of the light source and the distribution of the spores in the culture. In many

cultures only the more isolated spores will be oriented by light, the others all showing strongly the group orientation. The spores show the greatest individual differences in their relative sensitiveness to light and to the group stimulus. Of two spores lying within about 0.3 mm. of each other, one might be entirely oriented by the adjacent spore, while the other, apparently like it, would show only the action of the light stimulus. In many cases two such spores would seem to show a resultant effect of the two stimuli, so that both would be half turned toward each other, with both rhizoidal cells showing a tendency to take a direction away from the light at the same resultant angle (fig. 1).

The substance or condition originating in the activity of adjacent spores which has so powerful an effect in orienting the first cleavage plane and in determining which cell shall become the rhizoidal cell has no power to cause any chemotropism of the rhizoids after they are started. No rhizoid has been found to have its direction modified by the presence of other spores adjacent to it. In the absence of any light stimulus the rhizoids continue in the direction that they take originally from the spore.

Discussion

CHILD'S (**3**, **4**) metabolic gradient theory seems to offer the most satisfactory explanation of the power of environmental factors to establish the polarity of germinating spores. He has demonstrated in many marine plants and animals the existence of "axial susceptibility gradients" which he considers due to a decreasing rate in the metabolic processes from the apical to the basal or posterior end. Such a gradient would be established by differences in the rate at which oxidations and other reactions proceed, and by this disturbance of the equilibrium of the physiological mechanism would determine the basal and apical ends of the organism. CHILD'S explanation is as follows:

> Since extended experiment with the lower animals indicates that the degree of susceptibility to cyanides and to many other agents and conditions is in a general way, and within certain limits, a rough measure of metabolic activity or of certain fundamental metabolic processes, probably primarily the oxidations, these axial differences in susceptibility in the algae are regarded as indicating the existence of axial metabolic gradients. . . . . In the final analysis such a gradient is not self-determined by some sort of organization,

but arises as the result of the differential action of factors external to the protoplasm, cell, or cell mass acted upon. If, for example, an undifferentiated cell or cell mass is stimulated at some point by the action of a factor external to it, the resulting increase in metabolic activity is not limited to the region immediately affected, but a wave of change spreads or is transmitted over or through the protoplasm with decreasing energy, intensity, or physiological effectiveness, until, if the mass be large enough, it becomes inappreciable at a greater or less distance from the point of origin.

In the case of determination of the polarity of a spore by the direction of its illumination, it might be said, therefore, that a gradient is established within it by virtue of the fact that the oxidation reactions proceed more rapidly on the side receiving the greater amount of light energy. This side would become the apical end, if CHILD'S supposition is correct that the higher rate is toward the apex, or head, and that in the posterior parts, or in the rhizoids of algae, the rate is least. Thus it might be said that the disturbance of equilibrium within the spore due to the reception of unequal amounts of light energy over its surface produces an oxidation or metabolism gradient which establishes the polarity of the young plant. The spindle is oriented in some unknown way, and the less active of the two cells resulting from the first cleavage is the rhizoidal cell.

Why only the rays of the blue end of the spectrum should have this action is not clear. Possibly the cells exercise a selective absorption such as that described by BOVIE (2) as possessed by *Paramoecium* when acted upon by ultra violet light. Then the differences in the effects of monochromatic lights on *Fucus* spores would be due to differences in penetrating power rather than to "any action specific of wave length." Possibly the energy becomes available to the cell for its effect on oxidations through some photosensitive substance which responds only to the actinic rays.

CHILD has recently found a metabolic gradient or oxidation gradient in these germinating spores, and he finds that the region of highest susceptibility, which he takes to be the region of highest oxidation, is at first at the rhizoidal end, suggesting that the original effect of the light is an inhibition of reactions on the exposed side of the spores. A letter written to the author regarding this point contains the following:

As regards *Fucus*, I found the situation very interesting and very similar to that which exists in some of the lower animals. The egg shows a gradient as soon as polarity is determined, but as you suggested, it might be the region of highest susceptibility, which I believe to be the region of highest oxidation rate, is at first at the rhizoidal end. This makes it look as if the effect of light might be a differential inhibition rather than a differential stimulation. After five or six days, however, the susceptibility of the rhizoid decreases, and at the same time a new region of high susceptibility begins to appear at what is to be the apical end of the thallus, and this becomes and remains the most susceptible region of the plant, and from it a gradient of decreasing susceptibility extends basally to the base of the thallus. It looks as if the outgrowth of the rhizoid represented a rather brief period of high rate of oxidative activity, which soon slows down, and then the apical region of high rate arises, just as a bud, previously inhibited, arises or begins to develop when the activity of the growing tip which inhibited it is decreased. Of course these are at present merely suggestions by way of interpretation of the observed facts.

As for group orientation, it and the effect of light may have a common physiological basis. In both phenomena the controlling force may be gradients of increasing oxidation rates, but with different factors responsible. In the case of the light effect it may be the available energy speeding or retarding metabolic reactions where the wave lengths acting are such that they can be absorbed by the active substances of the cell; or in the case of group orientation, it may be available oxygen or other nutritive substance varying in amount on two sides of the spore as the result of the metabolic processes of adjacent spores.

We must conclude, however, that the attempts at partial explanation of these experiments and observations are far from satisfactory. The application of the oxidation gradient theory can only account for the later aspects of the polarity phenomena, the determination of the apical and basal ends of the germinating spore. The orientation of the first cleavage plane determined by orientation of the spindle of the dividing nucleus is visible evidence of forces existing within the cell, and the control of those forces by light energy and by chemical stimulation in a manner of which there is no hint, and the mechanics of which must remain obscure for the present.

Summary

1. A convenient method for obtaining monochromatic lights of equal intensity is the use of the thermopile and galvanometer

to obtain the relative intensity of the light transmitted by accurate color screens, and the adjustment of the distances of these screens from the light source such that the deflections of the indicator on the galvanometer scale are equal for each exposure of the thermopile screened by the light filters in turn.

2. The effective wave lengths in the establishment of the polarity of *Fucus* spores, the result of whose use for unilateral illumination is the same as that produced by white light (the orientation of the first cleavage plane perpendicular to the direction of the incident light with the cell on the darker side of the spore becoming the rhizoidal cell) are, with the intensity of strong diffused daylight, the shorter rays of the blue end of the spectrum of approximately 4000–5600 Ångstrom units. There is some evidence that ultra violet light can produce the same effect.

3. The negative phototropism of the rhizoids in monochromatic light is also primarily a function of the quality of the light, since, with equal intensity of illumination, the wave lengths of the red end of the spectrum are without effect, while those of 4000–5200 Ångstrom units produce the same phototropism produced by white light.

4. The term "group orientation" is suggested for the phenomenon of the orientation of the first cleavage plane of a dividing spore with reference to the position of adjacent spores, such that it is perpendicular to the direction of the center of a group or of a single spore within the effective radius, with the subsequent development of the cell on the side toward the source of stimulus as the rhizoidal cell.

5. This group orientation reported in other species is a conspicuous phenomenon in every culture of *Fucus inflatus*, the stimulus acting in such orientations being so strong that when spores are separated by as much as 0.2 mm. and often more, light stimuli as a rule fail to overcome it.

6. The chemical stimulus which orients the direction of the first cleavage plane and determines which cell shall become the rhizoidal cell in group orientations has no power to cause a chemotropism of the rhizoids.

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